Transport of Vitamin B_{12} in *Escherichia coli*: Common Receptor Sites for Vitamin B_{12} and the E Colicins on the Outer Membrane of the Cell Envelope

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The first step in the transport of cyanocobalamin (CN-B_{12}) by cells of *Escherichia coli* was shown previously to consist of binding of the B_{12} to specific receptor sites located on the outer membrane of the cell envelope. In this paper, evidence is presented that these B_{12} receptor sites also function as the receptors for the E colicins, and that there is competition between B_{12} and the E colicins for occupancy of these sites. The cell strains used were *E. coli* KBT001, a methionine/B_{12} auxotroph, and B_{12} transport mutants derived from strain KBT001. Colicins E1 and E3 inhibited binding of B_{12} to the outer membrane B_{12} receptor sites, and CN-B_{12} protected cells against these colicins. Half-maximal protection was given by CN-B_{12} concentrations in the range of 1 to 6 nM, depending upon the colicin concentration used. Colicin E1 competitively inhibited the binding of ^{57}Co-labeled CN-B_{12} to isolated outer membrane particles. Functional colicin E receptor sites were found in cell envelopes from cells of only those strains that possessed intact B_{12} receptors. Colicin K did not inhibit the binding of B_{12} to the outer membrane receptor sites, and no evidence was found for any identity between the B_{12} and colicin K receptors. However, both colicin K and colicin E1 inhibited the secondary phase of B_{12} transport, which is believed to consist of the energy-coupled movement of B_{12} across the inner membrane.

We have shown previously that the uptake of vitamin B_{12} by *Escherichia coli* consists of a rapid, initial phase followed by a slower, secondary phase (4, 5). The initial phase of uptake consists of binding of vitamin B_{12} to specific receptors which are firmly bound to the outer membrane of the cell envelope (14). The subsequent steps required to transfer the B_{12} from these receptors and move it across the inner membrane and into the interior of the cell constitute the secondary phase of uptake. At least one of these secondary steps is coupled to the energy metabolism of the cell. During the course of studies on the energy coupling processes involved in this transport system, the effects of colicin E1 upon B_{12} uptake were investigated. It has been shown in other laboratories that colicin E1 can inhibit some energy-coupled transport systems in *E. coli* (1, 6). In our work, it quickly became apparent that colicin E1, in addition to inhibiting the secondary

phase of B_{12} transport, inactivated the initial B_{12} binding sites. We have followed up this observation and have obtained evidence that the outer membrane receptor sites for vitamin B_{12} also serve as the receptor sites for the E colicins. These results are presented in this paper. Kadner and Liggins (9) also include some evidence indicative of genetic identity between the determinants for the outer membrane receptors for B_{12} and those for the colicin E receptors.

A preliminary report of this work was presented at the annual meeting of the American Society of Biological Chemists in Atlantic City, N.J., on April 18, 1973. (D. R. Di Masi, J. C. White, C. Bradbeer, Fed. Proc. 32: 600, 1973.)

MATERIALS AND METHODS

Radioactive compounds. Cyanocobalamin (CN-B_{12}), labeled with ^{57}Co, was obtained from the Amer-

sham/Searle Corp., Arlington Heights, Ill. L-proline-U-[14]C (200 mCi/mmol) and L-lysine-U-[14]C (210 mCi/mmol) came from ICN, Irvine, Calif., and Schwarz/Mann, Orangeburg, N.Y., supplied the L-leucine-U-[14]C (312 mCi/mmol).

Organisms. The bacterial strains used in this work were *E. coli* KBT001, a methionine/B₁₂ auxotroph of genotype F^- (*leu, pro, lysA, trp, purE, metE, str, lac*), and B₁₂ transport mutants derived from this strain. These strains have been described more fully previously (5, 15) and are listed in Table 1. Their genetic properties are presented in the accompanying paper (9). The cells were maintained on nutrient agar and, for experimental purposes, were grown on the minimal medium of Davis and Mingioli (3) supplemented with 0.5% glucose, adenine (40 μg/ml), and the required amino acids (50 μg/ml).

Colicin preparation. Colicins E1, E3 and K were prepared from cells of *E. coli* strains ML(COL E1⁺), CA38, and K235, respectively. The methods followed those described by Herschman and Helinski (8) for colicin E3, and included induction with mitomycin C (0.2 μg/ml), extraction of the cells with 1 M NaCl, and collection of the protein which precipitated between 15 and 50% saturated ammonium sulfate. The colicins were dissolved and diluted in colicin diluent, which was filter sterilized and which contained bovine serum albumin (2 mg/ml), 0.85% NaCl, 5 mM MgCl₂, and 0.5 mM CaCl₂.

Colicin assays. The colicins were assayed routinely by means of a plate assay (10). Plates of nutrient agar (containing 50 μg of streptomycin per ml) were overlaid with 0.7% nutrient agar (3 ml) containing about 10^8 cells from a sensitive strain of *E. coli* (usually KBT001). Samples (10 μliters) of 10-fold dilutions of the colicins were placed on the soft agar, and the plates were incubated overnight. The reciprocal of the highest dilution which gave a distinct inhibition of growth was used as a measure of the colicin titer.

Assay of CN-B₁₂ uptake. The methods used to measure uptake of CN-B₁₂ by whole cells of *E. coli*

have been described in detail previously (4) and consisted basically of the inclusion of radioactive CN-B₁₂ in the reaction mixtures, filtration of samples through membrane filters (0.45 μm pore size, Millipore Corp.), and liquid scintillation counting.

Amino acid uptake. Amino acid uptake methods were essentially the same as those described for CN-B₁₂ uptake, except that the reaction mixtures contained a ^{14}C-labeled amino acid instead of CN-B₁₂. Inhibition of amino acid uptake in whole cells of *E. coli* was used as another assay of activity of colicins E1 and K.

Assay of protein synthesis. In addition to the plate assay method, the activity of colicin E3 was also determined by measuring its ability to inhibit protein synthesis in cells of *E. coli*. Overnight cultures of an appropriate strain (usually KBT001) were used to inoculate final cultures in the minimal medium described above which was further supplemented with yeast extract (0.5 mg/ml). These cultures were grown to mid-log phase, then samples (5 ml) were transferred to small flasks in a water bath shaker at 37 C. After 10 min various amounts of colicin E3 were added and, after a further 10 min of incubation, 1 ml of the growth medium containing 0.25 μCi of L-[^{14}C]leucine was added to each flask. Shaking was continued for 10 min, and protein synthesis was stopped by pouring the samples into iced tubes which contained 0.5 mg of chloramphenicol in 0.5 ml of water. The samples were mixed and allowed to stand in ice for several minutes. Duplicate 2.5-ml samples were taken from each tube, added to 2.5 ml of 10% trichloroacetic acid, and incubated in boiling water for 20 min. The precipitated protein was collected on glass fiber filters (2.5 cm), which were then washed twice with 10 ml of 5% trichloroacetic acid (containing 0.2 mg of L-leucine per ml) and once with 10 ml of 95% ethanol. The filters were dried and counted in a liquid scintillation counter. Control experiments (experiments without colicin E3) showed that the incorporation of ^{14}C into precipitable protein was linear with time for about 20 min in this system.

Isolation of cell envelope particles from E. coli cells. These methods have been described previously (11, 14). Cells of the desired *E. coli* strain, from 1 liter of a late log-phase culture, were suspended in 200 ml of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer at pH 7.8, containing 1 mM ethylenediaminetetraacetic acid, and were mixed in a Sorvall Omnimixer. The cells were recovered by centrifugation at 0 C and were resuspended in 50 ml of the same buffer. After addition of about 1 mg each of pancreatic ribonuclease and deoxyribonuclease, the suspension was passed twice through an Aminco French pressure cell at 20,000 lbs/in². Magnesium chloride was added to give a final concentration of 2 mM, and any intact cells were removed by centrifugation at 3,000 × *g* for 5 min and at 6,000 × *g* for 7 min. The cell envelope particles were sedimented by centrifugation for 1 h at 2 C and 144,000 × *g*. Outer membrane particles were derived from these whole envelope preparations by selective solubilization of the inner membranes by using 10 mM KPO₄, pH 6.6, containing 2% Triton X-100 and 1 mM MgCl₂, and recentri-

TABLE 1. *Bacterial strains used*

E. coli strains	Genotype[a]	B₁₂ transport characteristics	
		Outer membrane B₁₂ binding sites	Energy-coupled secondary phase
KBT001	F^-, *leu, pro, lysA, trp, purE, metE, str, lac*	Present	Present
KBT026	As KBT001, but also *btuB*	Absent	Present[b]
KBT041	As KBT001, but also *btuA*	Present	Absent
KBT069	As KBT001, but also *btuB*	Absent	Present[b]

[a] For further details see Kadner and Liggins (9).

[b] Presumably present, but in the absence of the outer membrane B₁₂ binding sites, the secondary phase does not have access to the CN-B₁₂ in the medium.

fugation at 144,000 × *g* for 60 min, as described by Schnaitman (12).

Other methods. Protein was determined primarily by the method of Waddell (13).

RESULTS

Effects of colicins E1, E3, and K upon the uptake of CN-B_{12}. Figure 1 shows the effects of various amounts of colicin E1 upon the uptake of ^{57}Co-CN-B_{12} by whole cells of *E. coli* KBT001. In the absence of the colicin the normal biphasic pattern of B_{12} uptake was observed, which consisted of a rapid initial phase which was complete within the first minute, followed by a slower, secondary phase which was linear for about 30 min. The smallest amount of colicin E1 used, in the range of 5 to 50 killing units per cell, gave about 65% inhibition of the rate of the secondary phase of B_{12} uptake, with no detectable effect upon the initial phase. The largest amount of the colicin E1 used (50 to 500 killing units/cell), however, eliminated the
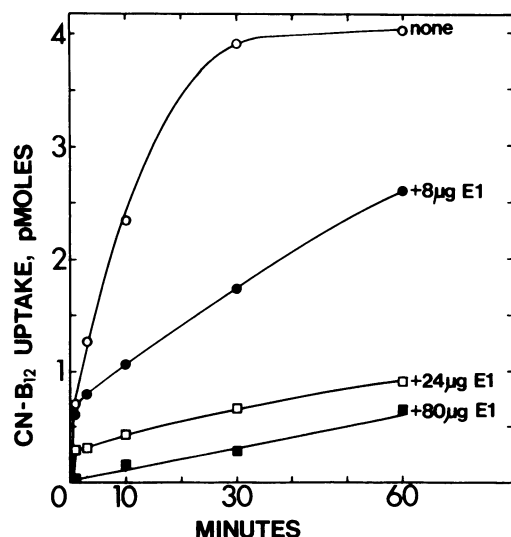

FIG. 1. *Effects of colicin E1 upon the uptake of ^{57}Co-CN-B_{12} by cells of E. coli KBT001. Each reaction mixture contained 0.1 M KPO_4, pH 6.8, 1% glucose, 4.5 nM ^{57}Co-CN-B_{12}, approximately 10^{10} cells, and various amounts of colicin E1, in a final volume of 10 ml. The reaction mixtures were preincubated at 37 C in a water bath shaker for about 15 min before addition of the colicin, and were then incubated 5 min longer before addition of the ^{57}Co-CN-B_{12}. Samples (1 ml) were removed after different time intervals. The amounts of colicin E1 used per reaction mixture were: none, O; 8 μg, ●; 24 μg, □; 80 μg, ■. From plate assays, the titer of the colicin E1 preparation was 10^5 per 16 μg of protein, giving an estimated 10^{10} to 6 × 10^{10} killing units per microgram of protein.*

initial phase of uptake; i.e., it inhibited the binding of CN-B_{12} to the B_{12} receptor sites on the outer membrane.

The effects of colicins E3 and K upon the uptake of ^{57}Co-CN-B_{12} were also examined in the same way, and these results are summarized in Table 2. The amounts of CN-B_{12} taken up in the first minute and between 1 and 20 min have been used to obtain estimates of the initial and secondary phases, respectively. Colicin K inhibited the energy-dependent, secondary phase of B_{12} uptake only, whereas colicin E3, in large amounts, inhibited both phases of uptake. We believe that the primary effect of colicin E3 in this system was to inhibit the initial B_{12} uptake, resulting in a concomitant decrease in the secondary phase. White et al. (14) have shown previously that an active initial phase is apparently necessary to provide B_{12} for the secondary uptake.

Sensitivity of E. coli strains KBT001, KBT026, KBT041, and KBT069 to colicins E1, E3, and K. The plate assay method was used to determine the sensitivity of the B_{12} transport mutant strains to the colicins E1, E3, and K (Table 3). Those strains (KBT001 and

TABLE 2. *Effects of colicins E_3 and K upon uptake of ^{57}Co-CN-B_{12} by cells of E. coli KBT001[a]*

Amt of colicin (μg/5 ml)	Initial phase of B_{12} uptake		Secondary phase of B_{12} uptake	
	Picomoles of B_{12} taken up	Percentage of total B_{12} uptake	Picomoles of B_{12} taken up	Percentage of total B_{12} uptake
E3				
None	0.75	100	3.87	100
0.13	0.73	97	3.63	94
1.3	0.54	72	4.00	103
13	0.02	3	0.82	21
K				
None	0.53	100	1.96	100
20	0.49	92	1.09	56
200	0.51	96	0.55	28

[a] Each reaction mixture contained 0.1 M KPO_4, pH 6.8, 1% glucose, 4.5 nM ^{57}Co-CN-B_{12}, cells of KBT001, and varying amounts of colicin E3 or K, in a final volume of 5 ml. There was a preincubation for 20 min at 37 C before addition of the ^{57}Co-CN-B_{12}. The colicins were added 10 min before the labeled B_{12}. Samples (1 ml) were removed after 1 and 20 min. The 1-min samples were used to give an estimate of the initial phase of B_{12} uptake, and the uptake between 1 and 20 min was used as a measure of the relative rate of the secondary phase. The results are expressed as picomoles of ^{57}Co-CN-B_{12} taken up and as percentages of the control values without colicin.

KBT041) which possessed an intact initial phase of B_{12} uptake (i.e., possessed functional outer membrane B_{12} receptor sites) were sensitive to colicins E1 and E3, whereas those strains (KBT026 and KBT069) which lacked functional B_{12} receptors were not sensitive to the E colicins. All of the strains were apparently equally sensitive to colicin K. Of particular interest was the observation that inclusion of

TABLE 3. *Sensitivity of various E. coli strains to colicins E_1, E_3, and K, in the presence and absence of CN-B_{12}*

Test strain	Colicin titer[a]					
	E₁		E₃		K	
	−B₁₂	+B₁₂	−B₁₂	+B₁₂	−B₁₂	+B₁₂
KBT001 ...	10⁵	10²	10⁴	10²	10³	10³
KBT026 ...	1	1	10		10³	10³
KBT041 ...	10⁵	10	10⁴	10	10³	10³
KBT069 ...	0	0	10		10³	10³

[a] Determined by means of the plate assay. Duplicate plates were set up, and one series contained 1 μM CN-B_{12} in the agar. The other series had no added B_{12}.
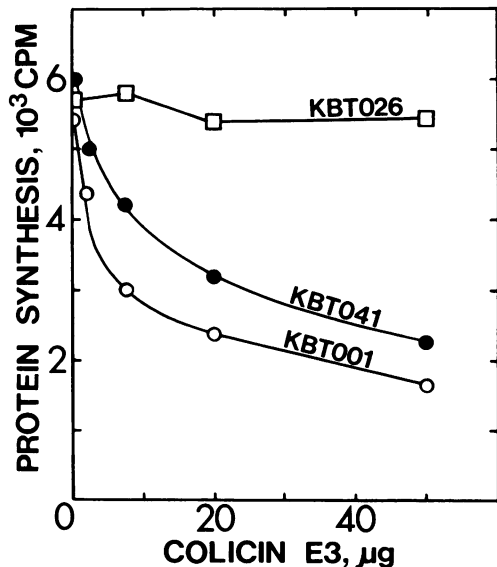

FIG. 2. *Sensitivity of E. coli strains KBT001, KBT026, and KBT041 to colicin E3. Inhibition of protein synthesis was used as a measure of the activity of colicin E3. The procedure is described fully in Materials and Methods. The results are plotted as counts per minute of L-[¹⁴C]leucine incorporated into protein at 37 C for 10 min versus the amount of colicin E3 per 5 ml of sample. The colicin E3 titer was 10⁵ per mg of protein.*
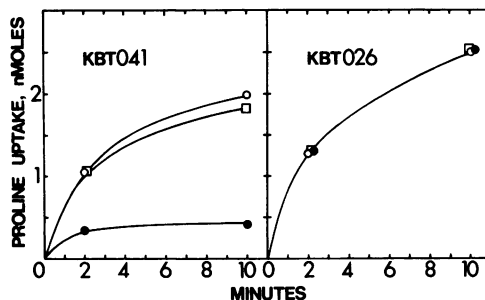

FIG. 3. *Effects of colicin E1 and CN-B_{12} upon the uptake of L-proline by cells of E. coli strains KBT041 and KBT026. Each reaction mixture contained 0.1 M KPO₄, pH 6.8, 1% glucose, approximately 3.3 × 10⁹ cells, and 10 μM L-[¹⁴C]proline in a final volume of 5 ml. There was an aerobic preincubation at 37 C for 20 min before addition of the [¹⁴C]proline. Where necessary, other additions included 0.5 μM CN-B_{12} (added at the beginning of the preincubation) and 1.6 μg of colicin E1 (added 5 min before the [¹⁴C]proline). Samples (1 ml) were removed for assay of proline uptake. Additions: none, □; colicin E1, ●; CN-B_{12} and colicin E1, ○. The amount of colicin E1 used was estimated to be in the range of 5 to 30 killing units per cell.*

CN-B_{12} (at a final concentration of 1 μM) in the nutrient agar protected sensitive strains against colicins E1 and E3, but not against colicin K.

Essentially the same results were obtained when other, more specific assays of colicin action were made. Figure 2 shows the effects of colicin E3 upon incorporation of L-[¹⁴C]leucine into protein by whole cells of three *E. coli* strains. No inhibition was obtained with cells of strain KBT026, which lack outer membrane B_{12} receptors. Similarly, Fig. 3 shows the effects of colicin E1 upon the uptake of L-[¹⁴C]proline by cells of strains KBT041 and KBT026. Again, there was no effect upon cells of strain KBT026. The uptake of proline was inhibited in cells of strain KBT041, but complete protection against the colicin E1 was obtained by inclusion of 1 μM CN-B_{12} in the reaction mixture.

Vitamin B_{12} was unable to protect cells from the action of colicin K. Colicin K inhibited the uptake of L-[¹⁴C]lysine by cells of strain KBT001, but CN-B_{12} had no detectable effect upon this inhibition (Fig. 4).

Assay of colicin receptors in cell envelopes from E. coli strains KBT001, KBT026, KBT041, and KBT069. The presence of colicin receptors in cell envelopes of the various *E. coli* strains was assayed by measuring the ability of such envelope preparations to neutralize the colicins. Tenfold dilutions of the colicins were incubated with cell envelope particles for 15 min at 37 C prior to assay by the plate tech-

nique (Table 4). Neutralization of the E colicins occurred with cell envelopes only from those strains which possessed functional B_{12} receptor sites on the outer membrane (i.e., KBT001 and KBT041, but not KBT026 or KBT069).

The neutralization of colicins E1 and K is also shown in Fig. 5. In these experiments, the envelope particles were removed by centrifugation (144,000 × g at 2 C for 30 min) after neutralization at 37 C, and the colicin content of the supernatant solutions was assayed by measuring their ability to inhibit the uptake of L-[^{14}C]lysine by cells of *E. coli* KBT001. Cell envelopes from strain KBT026, which lack the outer membrane B_{12} binding sites, were again shown to lack functional receptors for colicin E1. All of the strains tested possessed cell envelopes which were able to neutralize colicin K.

Interactions of vitamin B_{12} with the colicin systems. The ability of CN-B_{12} to protect sensitive cells against the action of the E colicins has been studied. Inhibition of lysine uptake was used as an index of colicin E1
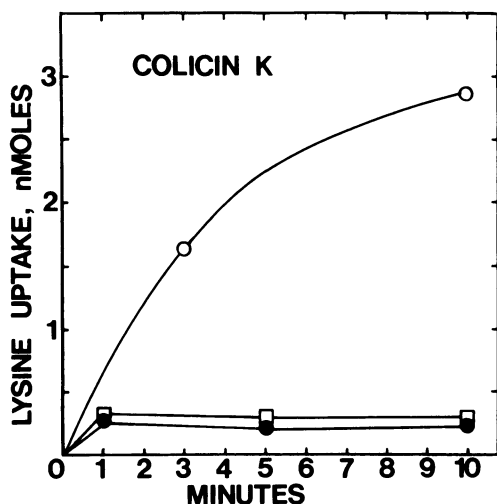
TABLE 4. *Neutralization of colicins E_1 and E_3 by cell envelope preparations from various strains of E. coli*[a]

Source of cell envelope	Colicin titer	
	E_1	E_3
None	10^2	10^2
KBT001	0	0
KBT026	10^2	10^2
KBT041	0	0
KBT069	10^2	10^2

[a] Tenfold dilutions of the colicins were made in colicin diluent or in colicin diluent containing 0.6 mg of cell envelope protein per 0.1 ml. After a preincubation at 37 C, 10 μliters of each dilution was spotted on an agar plate seeded with about 10^8 cells of *E. coli* KBT001, and colicin assays continued in the usual way.
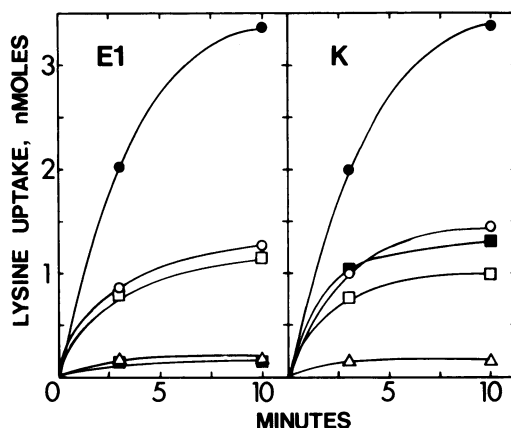

FIG. 5. *Neutralization of colicins E1 and K by cell envelope preparations from various strains of E. coli. Cell envelope particles were obtained from cells of E. coli strains KBT001, KBT026, and KBT041. Approximately 80 μg of colicin E1 or 200 μg of colicin K were incubated with protein (2 mg) of cell envelopes in colicin diluent reaction mixtures (1 ml) at 37 C for 15 min, and were then centrifuged at 144,000 × g for 30 min at 2 C. The supernatant solutions were assayed for colicin E1 and K by measuring the inhibition of [^{14}C]lysine uptake in cells of E. coli KBT001, exactly as described for Fig. 4. Additions: none, ●; untreated colicins, △; colicin which had been treated with cell envelopes from E. coli strains KBT001, ○; KBT026 ■; and KBT041 □.*

FIG. 4. *Effects of colicin K and CN-B_{12} upon the uptake of L-[^{14}C]lysine by cells of E. coli KBT001. Each reaction mixture contained 0.1 M KPO_4, pH 6.8, 10 mM $MgSO_4$, 1% sodium succinate, 10 μM L-[^{14}C]lysine, and approximately 3 × 10^9 succinate-grown cells in 5 ml final volume. The incubation was aerobic at 37 C, and there was a 20-min preincubation before addition of the [^{14}C]lysine. Where necessary, 1.2 μM CN-B_{12} (added at the beginning of the preincubation) and 200 μg of colicin K (added 10 min before [^{14}C]lysine were included in the reaction mixtures. Samples (1 ml) were removed at different times. The colicin K titer was about 2 × 10^4 per mg of protein. Additions: none, ○; colicin K, ●; CN-B_{12} and colicin K, □.*

activity. The amount of colicin E1 used was that which gave about 70% inhibition of the rate of lysine uptake by cells of *E. coli* KBT041. This strain was selected because it has no secondary phase of B_{12} uptake, and the B_{12} taken up remains associated with the cell surface. The degree of protection conferred by varying levels of CN-B_{12} against colicin E1 in this system was

measured. The results are plotted in Fig. 6 as the percentage of protection versus the CN-B_{12} concentration in the reaction mixtures. The rate of lysine uptake in the presence of colicin E1 and without any B_{12} was 0%, and the rate of lysine uptake in the absence of the colicin was 100%. The inset (Fig. 6) shows a double reciprocal plot of the same data, and an apparent K_s for CN-B_{12} of about 1 nM was obtained. This value is only slightly above that which was previously determined for the B_{12} receptor sites (14).

In a similar experiment the effects of the CN-B_{12} concentration upon colicin E3 activity were studied. Inhibition of the rate of protein synthesis by cells of E. coli KBT001 was used as a measure of colicin E3 activity. In Fig. 7 the percentage of protection against colicin E3 is plotted against the concentration of CN-B_{12}. Half-maximal protection was given by about 6 nM CN-B_{12}.

Further evidence for the interaction of vita-

FIG. 7. Effects of CN-B_{12} concentration upon the activity of colicin E3. The protein synthesis assay with cells of E. coli KBT001 was used to measure the activity of colicin E3. Where necessary, the reaction mixtures contained 20 µg of colicin E3 per 5 ml of sample. The results are plotted in the same way as described for Fig. 6, as the percentage of protection against colicin E3 conferred by each concentration of CN-B_{12}. The inset shows a double reciprocal plot of the same data.

min B_{12} and colicin E1 at the same outer membrane sites is presented in Fig. 8. In this experiment the effects of colicin E1 upon the binding of ^{57}Co-CN-B_{12} to outer membrane particles from E. coli KBT001 were measured at 0 C, by using the membrane filtration assay. The concentrations of colicin E1 and of CN-B_{12} were both varied. The lines of best fit in the double reciprocal plot (inset, Fig. 8) were found by means of linear regression analyses of the data. The results are consistent with the view that colicin E1 is a competitive inhibitor of CN-B_{12} binding by the outer membrane particles. A similar experiment at 4 C, in which the binding of ^{57}Co-CN-B_{12} was measured by equilibrium dialysis, gave essentially the same results (data not shown).

DISCUSSION

Previous studies have shown that the uptake of vitamin B_{12} by cells of E. coli consists of two distinct, sequential phases (4). The first of these is rapid binding of the B_{12} to specific receptor sites which are firmly attached to the outer membrane of the cell envelope (14). There are approximately 200 such sites per cell. These sites have an apparent physiological function in enabling B_{12}-methionine auxotrophs to grow more rapidly than mutants which lack these
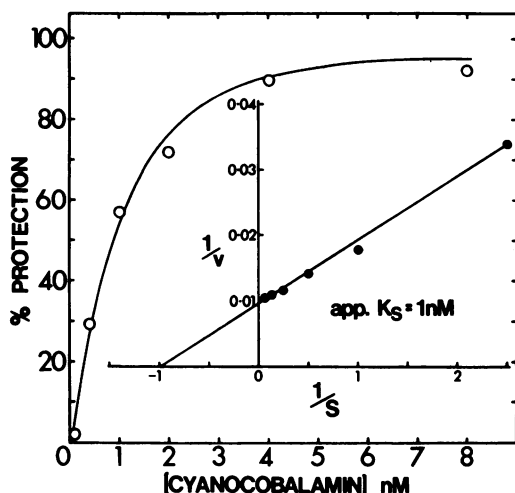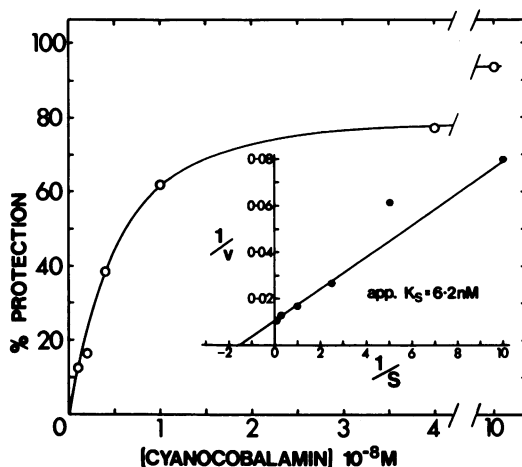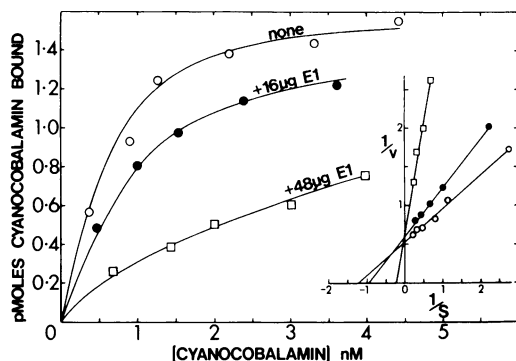

FIG. 6. Effects of varying concentrations of CN-B_{12} upon the activity of colicin E1. Inhibition of L-[^{14}C]lysine uptake in cells of E. coli KBT041 was used as a measure of colicin E1 activity. The procedure used was the same as that described for Fig. 4. Where necessary, the reaction mixtures contained 8 µg of colicin E1 and various concentrations of CN-B_{12}. The concentrations of CN-B_{12} in the reaction mixtures are plotted against the percentage of protection against colicin E1 given by each B_{12} concentration. The percentage of protection is defined as $100 (V_{E1B_{12}} - V_{E1})/(V_{control} - V_{E1})$ where $V_{control}$ = 10 min lysine uptake in the absence of both B_{12} and colicin E1; V_{E1} = 10 min lysine uptake in the presence of colicin E1; and $V_{E1B_{12}}$ = 10 min lysine uptake in presence of both colicin E1 and CN-B_{12}. The inset shows a double reciprocal plot of the same data, in which the line of best fit was found by a linear regression analysis.

FIG. 8. *Effects of colicin E1 upon the binding of* ^{57}Co-CN-B_{12} *by outer membrane particles from E. coli KBT001. Reaction mixtures (1 ml), containing 10 mM KPO$_4$, pH 6.6, 0.1 mM KCN, 0.25 mg of protein of outer membranes, and various amounts of* ^{57}Co-CN-B_{12} *and colicin E1, were incubated in an ice bath for 1 h, and were then filtered through a membrane filter. The filters were washed with 10 ml of 10 mM KPO$_4$, pH 6.6, dried, and counted. For each concentration of colicin, the amount of* ^{57}Co-CN-B_{12} *bound is plotted against the CN-B$_{12}$ concentration. The inset is a double reciprocal plot of the same data. The amounts of colicin E1 used were: none, O; 16 μg, ●; and 48 μg, □.*

sites (media containing low B_{12} concentrations and no methionine) (9). This initial binding of B_{12} to these outer membrane receptors is not dependent upon coupled energy metabolism (4). The secondary phase of B_{12} uptake, however, is coupled to the cell's energy metabolism, and consists of the transfer of the B_{12} from the outer membrane receptors across the inner membrane and into the cell's interior. Colicins E1 and K are known to inhibit the energy metabolism of aerobically grown cells of *E. coli* and to inhibit the energy-coupled transport of amino acids and of β-galactosides (1, 6).

In the experimental work described in this paper, we were initially interested in determining whether colicins E1 and K inhibited the energy-dependent phase of B_{12} transport in *E. coli*. Colicin E1 was found to inhibit the energy-coupled secondary phase of B_{12} uptake (Fig. 1). Of some interest, however, was the observation that this colicin also inhibited the binding of CN-B_{12} to the outer membrane B_{12} receptors. From the results of further experimentation, presented here and by Kadner and Liggins (9), we have concluded that the outer membrane sites, which bind vitamin B_{12} and function as part of the B_{12} transport system, also serve as the receptors for the E colicins. The evidence that supports this view is summarized below. Those strains of *E. coli* that lacked outer

membrane B_{12} binding sites also lacked receptors for colicins E1 and E3. Kadner and Liggins (9) have shown that approximately 90% of the cell strains, which were isolated on the basis of a lack of sensitivity to colicin E1, lacked functional B_{12} receptors on their outer membranes. The other 10% of these mutant strains were presumably tolerant, rather than resistant, to this colicin. Kadner and Liggins also showed that the B_{12} receptor locus, *btuB*, is at the same position on the *E. coli* chromosome as that previously shown for the locus *bfe* of the colicin E receptors. The number of B_{12} binding sites per cell (180–220) of *E. coli* KBT001 (14) is essentially the same as the number (220) of colicin E3 receptor sites which Sabet and Schnaitman (10a) found per cell of *E. coli* K-12 C600. We have also shown that vitamin B_{12} and the E colicins apparently compete for the same sites on the *E. coli* cell surface. Thus, vitamin B_{12} was able to protect sensitive cells against colicins E1 and E3. Half-maximal protection was given by CN-B_{12} concentrations which were within one order of magnitude higher than the K_s for CN-B_{12} of the B_{12} receptor sites. Colicin E1 competitively inhibited B_{12} binding by outer membrane preparations from *E. coli* KBT001 (Fig. 8). The same correlations between sensitivity to the E colicins and the presence of functional B_{12} receptors were also observed in experiments which measured the inhibition of amino acid uptake by colicin E1, and the inhibition of protein synthesis by colicin E3. There was no correlation between the presence of B_{12} receptors and sensitivity of cells to colicin K, although colicin K did inhibit the energy-coupled secondary phase of B_{12} transport. The results of some control experiments, in which we were unable to detect any direct interaction between vitamin B_{12} and the E colicins, are consistent with our view that B_{12} and these colicins interact via competition for common receptor sites. Thus CN-B_{12} did not inactivate colicins E1 and E3, and these colicins did not bind ^{57}Co-CN-B_{12} (data not shown).

Although vitamin B_{12} and the E colicins use the same outer membrane receptors, it seems likely that they do not share a common system for crossing or interacting with the inner membrane. This conclusion is based on the observation that cells of *E. coli* KBT041 (a strain which lacks the energy-coupled secondary phase of B_{12} transport but possesses functional outer membrane B_{12} binding sites) are sensitive to colicins E1 and E3.

The colicin E receptors on the *E. coli* cell envelope apparently also serve as receptor sites for the bacteriophage BF23 (2, 7). It might be

expected, therefore, that vitamin B_{12} would protect sensitive cells against this bacteriophage, but we have not yet investigated this possibility. The utilization of the same receptor sites by vitamin B_{12}, bacteriophage BF23, and the E colicins does not necessarily mean that all of the components of these receptors are shared. Sabet and Schnaitman (10a) have obtained some evidence which indicates that the colicin E1 receptors require some additional constituent which is not required by the E3 receptors.

The possession of B_{12} receptors in the outer membrane of the *E. coli* cell envelope evidently confers a physiological advantage upon these cells, whereas colicin receptors per se are clearly potentially dangerous. The association of these disparate functions within a single structural element offers a possible rationale for the evolutionary retention of the disadvantageous component. It will be of interest to see whether the receptors for other colicins and other bacteriophages are necessary components of some other physiologically advantageous systems.

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